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Ribosomes & Translation

1316-Pos Board B208

Initiation Factor 3 (IF3) does not have any Role in the Release of Messenger RNA and Transfer RNA from the Post-Termination Complex during the Ribosome Recycling Reaction Catalyzed by EF-G/GTP and RRF (Ribosome Recycling Factor)

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At the end of translation of an ORF, the ribosome meets the termination codon and peptides are released by release factors 1, 2 and 3. The resulting post-termination complex (PoTC) is characterized to have one tRNA and mRNA per ribosome. We showed that it is disassembled by RRF and EF-G/GTP into mRNA, tRNA, and subunits (JBC, 1973, 248, 7580). On the other hand, others claimed that RRF and EF-G split the ribosome but do not release mRNA or tRNA. IF3 was claimed to be necessary to release mRNA and/or tRNA from the complex of 30S/tRNA/mRNA. This wrong conclusion is due to the use of short ORF with strong SD sequence (Mol Cell., 2005, 18, 675; Mol Cell., 2005, 18, 403). We use more natural PoTC which is free of the influence of the SD sequence, and show here that IF3 has no role in the release of mRNA and tRNA from the PoTC. We (RNA, 2005, 8, 1317) and others showed that splitting of subunits occurs due to RRF and EF-G. It has been known that IF3 prevents association of subunits. However, its effect on the rate of ribosomal splitting by EF-G/RRF remains to be elucidated. In this paper, we will present the results which shed lights on this question.

1317-Pos Board B209

Monitoring Translation with Modified MRNAs Strategically Labeled with Isomorphic Fluorescent Nucleosides

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To kinetically monitor translation-related events at a nucleotide resolution, a series of mRNAs, site-specifically modified at key codon positions with new emissive and responsive isomorphic nucleotides (th**C**, th**U**, th**G** - work with th**A** is commencing) have been studied. mRNAs modified with th**C**, th**U** and th**G** (excluding th**U**2 in the start codon) form an initiation complex (70SIC) and show significant spectral changes between the free and 70SIC-bound fluorescent mRNAs. All emissive mRNAs tested containing labeled nucleotides in the second codon facilitate aa-tRNA A-site binding [as part of an aa-tRNA.EF-Tu.GTP ternary complex (TC)] and pretranslocation (PRE) complex formation, although with somewhat different relative efficiencies, and all PRE complexes can be translocated via addition of EF-G.GTP to form postranslocation (POST) complexes. In most cases spectral differences are seen on conversion of 70SIC to PRE complexes and PRE complexes to POST complexes, allowing measurement of the kinetics of such conversions by changes in the fluorescence of labeled mRNAs. Preliminary comparison of tRNA A-site binding kinetics, as monitored by the fluorescent mRNAs, with other measures of PRE complex formation indicate that TC binding to the ribosome is faster than codon-anticodon interaction, which in turn is somewhat faster than tRNA accommodation. These preliminary observations not only show excellent performance of mRNAs modified with our emissive RNA alphabet, but also clearly demonstrate their potential to illuminate the formation and disappearance of discrete intermediates in the polypeptide elongation cycle.

1318-Pos Board B210

Dependence of Protein Synthesis on TRNA Concentrations

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During protein synthesis, a ribosome moves along a messenger RNA (mRNA) and translates it codon by codon by binding and recognizing the appropriate aminoacylated transfer RNAs (aa-tRNAs). We developed a stochastic theory for this process of translation elongation and determined the dependence of elongation speed on codon sequence as well as on the concentrations of ribosomes and and aa-tRNAs. We find that the variation of a single aa-tRNA concentration leads to three distinct regimes of protein synthesis. In the depletion regime characterized by low aa-tRNA concentrations, the ribosomal peptide synthesis rate follows a general Michaelis-Menten law. Furthermore, we find a remarkably broad range of intermediate aa-tRNA concentrations, for which translation is very stable and the ribosomal peptide synthesis rate is essentially constant. For large concentrations, crowding of aa-tRNAs and the competition between non-cognate and cognate aa-tRNAs strongly impede protein synthesis. Our results provide new insights into the process of protein synthesis and the role of aa-tRNA concentrations therein. Knowing the influence of aa-tRNA concentrations on translation is pivotal to further progress in understanding and controlling protein synthesis, e.g., in tRNA over-expressing cancer cells as well as in cell-free expression systems and organisms bioengineered for high yield protein production.

1319-Pos Board B211

Structural Complementation of a Monomeric Ribosomal-Inactivating Protein

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Exotoxin A (ETA) from the Gram-negative bacterium *Pseudomonas aeruginosa* is a ribosomal-inactivating protein (RIP) that acts by ADP-ribosylation of the post-translationally modified histidine, diphthamide, in elongation factor 2. Apoptotic cell death typically ensues upon intoxication. Distinct from toxins with multiple subunits, ETA is a single-chain protein with three functional domains. The catalytic domain (domain III, or PE3) of pseudomonal exotoxin A is a 213-residue monomer with extensive sequence and structural homology with the A chain of diphtheria toxin and the more recently-characterized cholix toxin from *Vibrio cholerae*. We are interested in developing split fragments of PE3 capable of structural complementation. Based on B-factors in the crystal structure of PE3, we have identified several disordered loops as candidates for severance, none of which are spatially close to the active site. We have produced and characterized fusion constructs each consisting of a split fragment and one subunit of a heterospecific, antiparallel coiled-coil. We report one such pair that remains inactive individually but undergoes complementation to yield a functionally active RIP *in vitro* and we are characterizing its biophysical properties. To our knowledge, this pair of split PE3 fragments represents the first example structural complementation of a monomeric protein toxin and may have significant biotechnological and therapeutic applications. Our ultimate goal is to engineer a cellular toxin with multiple specificities to be achieved by conditional co-expression inside a target cell.

1320-Pos Board B212

Observing Prokaryotic Translation Elongation in Real-Time using Single-Molecule Fluorescence

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Translation elongation is a highly dynamic and energy intensive process where the ribosome must processively decode each codon encoded by the mRNA and add the corresponding amino acid via the correct tRNA. The process achieves a delicate balance between speed, proceeding at up to 20 amino acids per second in vivo, and accuracy, with an error rate of 1 error in 10,000 amino acids, while translating mRNAs with potentially complex sequences and structures. The ribosome must undergo significant conformational changes during elongation and move the mRNA in precise codon-sized steps to maintain reading frame. Accordingly, this process is the target of many clinically important antibiotics which increase the energy barrier to various steps of elongation in order to inhibit peptide synthesis. Nevertheless, the detailed effects of those antibiotics are challenging to probe using conventional biochemical techniques over the stochastic and processive process of elongation. To overcome the limited parameters simultaneously observable by one single-molecule experiment, we employed multiple single-molecule fluorescence techniques to probe elongation in real-time over multiple codons from multiple perspectives. We observed the mechanistically distinct effects of various aminoglycosides and other antibiotics on the steps of elongation. We additionally are extending our single-molecule methods from simple model mRNAs to ones with realistic coding sequences using a commercially available in vitro translation system. This opens the door for observing elongation dynamics over complex mRNA sequences to observe how complex mRNA sequences, such as secondary structures, internal Shine-Dalgarno-like sequences, or frame-shifting sequences, affect elongation dynamics. These studies demonstrate the power and potential of real-time single-molecule fluorescence techniques in dissecting the mechanisms of complex processes during translation and probing the global energy landscape of elongation under various conditions.